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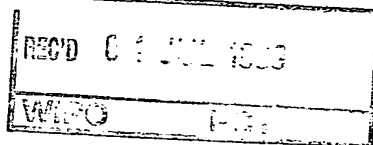
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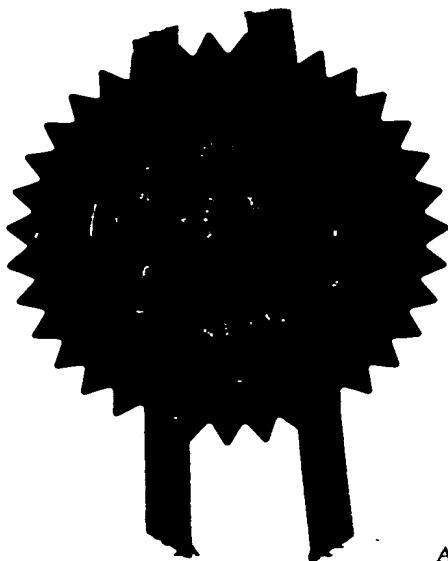


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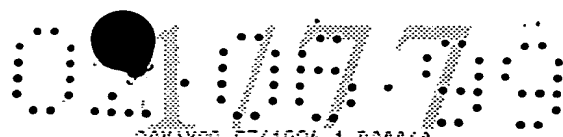
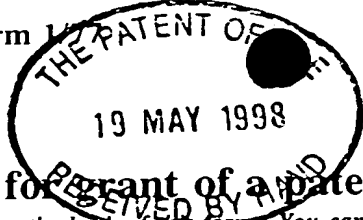
Andrew Gersey

Dated

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3. Full name, address and postcode of the or of each applicant (underline all surnames)	ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE P. ANGELETTI SPA VIA PONTINA KM. 30.600 00040 POMEZIA (ROMA) ITALY		
Patents ADP number (if you know it)			
If the applicant is a corporate body, give the country/state of its incorporation	IT	7439219001	
4. Title of the invention	MIMOTOPES OF HYPERVARIABLE REGION 1 OF THE E2 GLYCOPROTEIN OF HCV AND USES THEREOF		
5. Name of your agent (if you have one)	MEWBURN ELLIS		
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Claim(s) 20

Abstract 1

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MIMOTOPES OF HYPERVARIABLE REGION 1 OF THE
E2 GLYCOPROTEIN OF HCV AND USES THEREOF

The present invention is concerned with peptides,
specifically peptides which are mimotopes of the hypervariable
5 region 1 (HVR1) of the putative envelope protein E2 of
hepatitis C virus (HCV). Employing a combination of techniques
the present inventors have devised a large number of peptides
with sequences based on consensus analysis of naturally
occurring HVR1 sequences and experimental determination of
10 cross-reactivity to antibodies against different isolates, none
of which peptides occurs in nature. The peptides are
individually useful in raising and obtaining antibodies, for *in*
vitro (e.g. diagnostic) and *in vivo* purposes, and libraries of
peptides are useful in identifying peptides of particular
15 cross-reactivity with antibodies able to bind a plurality of
HVR1's of different HCV strains. Peptides may be used in
themselves or as part of fusion proteins, for instance in
recombinant HCV E2 polypeptides, which may be incorporated into
recombinant HCV particles.

20

The HVR1 region of HCV is the most variable antigenic
fragment in the whole viral genome and is mainly responsible of
the large inter and intra-individual heterogeneity of the
infecting virus. It contains a principal neutralization

epitope and has been proposed as the major player in the mechanism of escape from host immune response. Since anti-HVR1 antibodies are the only species shown to possess protective activity up to date, the development of an efficient prevention therapy is a very difficult task.

In devising the present invention, the inventors approached the problem of the HVR1 variability by deriving a consensus profile from more than two hundred HVR1 sequences from different viral isolates and used this consensus as a template for generating a vast repertoire of synthetic HVR1 surrogates. These were provided as fusions to the major coat protein VIII of M13 bacteriophage for display on the surface of bacteriophage particles. This library was affinity selected using many different sera from infected patients. Phage were identified which displayed high frequency of reactivity with patients' sera, but not with sera from uninfected controls. The selected sequences were shown to bind serum antibodies cross-reacting with a large panel of peptides reproducing the HVR1 from natural HCV variants.

20 In these "mimotopes" was identified a sequence pattern responsible for the observed cross-reactivity. When injected in experimental animals, the mimotopes with the highest cross-reactivity induced antibodies able to recognise the same panel of natural HVR1 variants.

Hepatitis C virus (HCV) is the major etiologic agent of both blood-transfusion-associated and sporadic non-A non-B hepatitis worldwide, with an estimated prevalence between 0.4 and 2% in the blood donor population (Choo et al., 1989). HCV infection leads to viral persistence and chronic disease in at least 70% of cases, among which a significant proportion eventually develops cirrhosis and hepatocellular carcinoma (for a review see H. Alter, 1995). In spite of the availability of reliable serological tests for HCV diagnosis, community-acquired infection is still common and causes significant morbidity and mortality worldwide (Mast and Alter, 1993). In addition, interferon treatment, which is the only anti-viral therapy available at the moment, is effective only in 20-30% of patients (Fried and Hoofnagle, 1995). Thus, development of an HCV vaccine represents a high priority project in the field.

The high frequency with which the virus establishes a persistent infection, despite a wide array of humoral and cell-mediated host immune responses, raised in the past some concerns about the existence of a protective immunity against HCV (Farci et al., 1992). As a matter of fact, protective immunity against challenge with homologous virus could be induced by vaccination of chimpanzees (the only other species susceptible to HCV infection) using recombinant forms of the putative envelope proteins E1 and E2 (Choo et al., 1994).

However, it remains to be established how effective this response would be against heterologous viral inocula.

HCV exists in the bloodstream of infected patients as a quasispecies (Weiner et al., 1991; Martell et al., 1992; 5 Martell et al., 1994; Kurosaki et al., 1994; Bukh et al., 1995) which fluctuates during the course of the disease mainly as a result of immune pressure (Weiner et al., 1992; Kato et al., 1993; Kojima et al., 1994; Shimizu et al., 1994; van Doorn et al., 1995; Weiner et al., 1995). The emerging view is that 10 chronic infection by HCV is not due to lack of humoral or cellular responses, but rather to such responses being rendered ineffective by the high mutation rate of the virus which leads to the emergence of escape variants.

The existence of neutralising antibodies and their role in 15 protection from viral infection was documented by *ex vivo* neutralization of a pedigreed viral inoculum prior to injection into chimpanzees (Farci et al., 1994). This notwithstanding, neutralising antibodies were isolate-specific and seemed to be able to block only viral variants which were present before the 20 onset of the corresponding humoral response (Farci et al., 1994). Even if the specificity of such neutralising antibodies is not well defined, both immunological and molecular evidence indicate that epitopes recognised by neutralising antibodies are localised in the hypervariable region 1 (HVR1) of the HCV

genome (Farci et al., 1994). This consists of the N-terminal
 27 amino acids of the E2 glycoprotein, the most variable region
 of the whole HCV polyprotein (Weiner et al., 1991). Direct
 proof for the role of anti-HVR1 antibodies in virus clearance
 5 came recently from ex vivo neutralization experiments. A
 rabbit anti-HVR1 hyperimmune serum raised against the
 predominant variant of an infectious HCV inoculum abolished its
 infectivity in one chimp, and partially protected a second
 animal by blocking propagation of the major variant present in
 10 the inoculum (Farci et al., 1996).

Thus, the evidence is that the HVR1 contains a principal
 neutralization determinant for HCV, and that it should
 constitute an essential component of an acellular HCV vaccine
 if one could surmount the problem of its variability. Relevant
 15 to this issue is the observation that anti-HVR1 antibodies from
 human sera display some degree of cross-reactivity to different
 HVR1 variants (Scarselli et al., 1995).

W094/26306 (Chiron Corporation) discloses an attempt at
 identifying a consensus sequence within the HVR1 of HCV, based
 20 on sequence comparison on the 90 strains said to have been
 known as of 12 May 1993. The disclosed formula is of a peptide
 including the following sequence: aa1-aa2-aa3-aa4-aa5-aa6,
 wherein aa1 is S, G, A, D, K, R or T; aa2 is L, F, I, M or W;
 aa3 is F or L; aa4 is any amino acid; aa5 is any amino acid;

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and aa6 is G or A; with the proviso that the motif is not contained within a 31 amino acid sequence of a naturally occurring E2HV domain of an HCV isolate known as of May 12, 1993. In a further embodiment, aa7 is present and attached to 5 aa6; aa7 being A, P or S. The 6 amino acid motif represents around 55,000 different sequence. The 7 amino acid motif represents around 165,000 different sequences.

Aspects of the present invention are based in part on a 10 close inspection of the variability in HVR1 revealing that some positions of the HVR1 are less variable than others, suggesting that the actual structural, and immunological variability is more limited than that suggested by the heterogeneity in primary sequence. The invention is concerned in various 15 aspects with providing "synthetic variants" of the HCV HVR1 which are immunologically similar to a plurality, preferably a great number of natural HVR1 variants and, therefore, may be used to induce neutralising antibodies which cross-react with different HCV variants, preferably most or all. As explained 20 further below, the formulae arrived at for peptides of the present invention differs from that provided in WO94/26306, and is based on actual cross-reactivity scoring rather than just sequence comparison.

Phage displayed peptide libraries offer the unique chance to rapidly survey large collections of peptidic sequences (10^8 or more) through a cyclic selection/rescue/amplification procedure. They allow identification of ligands for any type of ligate ranging from linear peptides to folded protein domains, and even carbohydrates (Cortese et al., 1994, Cortese et al., 1996). These ligands are true mimotopes as they do not necessarily share the same amino acid sequence of the original epitope, but they mimic its binding properties. A strategy for the identification of disease-specific phage-displayed mimotopes was reported previously, which avails itself only of clinically characterized sera from immune and non immune individuals (Folgori et al., 1994, hereby incorporated by reference). Furthermore, disease-specific mimotopes proved to be good immunogenic mimics of the natural antigen as they were able to induce a specific immune response to the natural antigen when injected into different animals (Folgori et al., 1994, and Meola et al., 1995, (both hereby incorporated by reference) Prezzi et al., 1996, Mecchia et al., 1996). Thus, phage libraries may be used as a source of artificial ligands recognised by disease-specific antibodies, with the advantage that additional desirable features can be built-in, providing that they can be selected for during library enrichment.

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In making the present invention, the inventors approached the problem of the HVR1 variability by generating a vast repertoire of HVR1 surrogates as fusion to the major coat protein (pVIII) of bacteriophage M13. Using the power of
5 selection and many sera from clinically characterized HCV infected individuals peptides were isolated which revealed to be good antigenic and immunogenic mimics of a large number of naturally occurring HCV variants.

10 Experimental details are provided below.

In accordance with various aspects of the present invention there are provided libraries of peptides containing large numbers of different peptides, individual peptides which
15 contain epitopes cross-reactive with a plurality of HCV HVR1 epitopes, and mixtures of different such peptides.

One aspect of the present invention provides a library of peptide conforming with the following consensus profile:

20

Q	T	H	V	T	G	G	S	A	A	R	T	T	S	G	L	T	S	L	F	S	P	G	A	S	Q	N
T		T	T	V			V	Q	G	H	A	A	H	S		V	G		R	L		P	K		K	
			R				Q	V	S		Q	V	R	R		R			S		S	Q				
																			Q							

This profile represents a total of 9×10^7 individual sequences, i.e. a number very close to the upper practical limit (about 10^8) of current DNA cloning and transformation techniques. As described below, this consensus profile was used for the

5 construction of a 27aa peptide library by cloning a degenerated synthetic oligonucleotide as a fusion to the 5' end of the gene coding for the major coat protein (pVIII) in a phagemid vector for M13. The library was extensively screened using human sera, and more than one hundred different clones (mimotopes) were
10 selected for their characteristic to specifically recognise human anti HCV-HVR1 antibodies. Nearly all these mimotopes have different amino acid sequences and none of them could be found to correspond to published (up to January 98) natural HVR1.

15 In a preferred embodiment of a peptide library according to the present invention there are at least about 10^5 different peptides present, preferably at least about 10^6 different peptides, more preferably at least about 10^7 , e.g. about 9×10^7 different peptides.

20 A library of peptides may be displayed on the surface of bacteriophage, particularly filamentous bacteriophage such as fd or M13, for instance as fusions with the major coat protein (pVIII) of such bacteriophage. Phage display of peptides is standard in the art and its power lies in the fact that

bacteriophage particles are constructed so that packaged within each particle is nucleic acid encoding the peptide displayed on its surface. Following selection of phage particles displaying a peptide of interest, such as a peptide able to bind one or 5 more antibodies (e.g. antibodies able to bind a number of epitopes of HVR1 of different strains of HCV), the nucleic acid encoding the displayed peptide can be retrieved and used in production of further peptide with that amino acid sequence.

10 In the experimental work described below, the inventors tested mimotopes in a library according to the present invention with a panel of human sera, and individual mimotopes were characterised as having a different overall frequency of reactivity with the sera. The 24 clones that only reacted with 15 less than 3 sera were defined as "weak" while the 27 reacting with more than 11 sera were defined as "strong".

Statistical analysis of the consensus sequences of "strong" and "weak" clones, lead to the discovery of a sequence motif in the HVR1 that is correlated with high frequency of 20 reaction with human sera, crossreactivity with human anti HVR1 antibodies and induction of highly crossreactive sera in experimental animals.

Peptides according to the present invention, and mixtures

thereof, may be defined as follows, further explanation of which is given below in the experimental section:

(1) - A library of peptides fully described by the following 5 formula ("Formula I"):

```

Q T H V T G G S A A R T T S G L T S L F S P G A S Q N
T   T T V       V Q G H A A H S   V G       R L   P K   K
      R       Q V S   Q V R R       R       S   S Q

```

10

Q

which may be written as

(aa1)T(aa3)(aa4)(aa5)GG(aa8)(aa9)(aa10)(aa11)(aa12)(aa13)(aa14)
(aa15)L(aa17)(aa18)LF(aa21)(aa22)G(aa24)(aa25)Q(aa27)

15 wherein aa1 is Q or T; aa3 is H, T or R; aa4 is V or T; aa5 is
T or V; aa8 is S, V or Q; aa9 is A, Q or V; aa10 is A, G or S;
aa11 is R or H; aa12 is T, A or Q; aa13 is T, A or V; aa14 is
S, H or R; aa15 is G, S or R; aa17 is T or V; aa18 is S, G or
R; aa21 is S or R; aa22 is P, L, S or Q; aa24 is A, P or S;
20 aa25 is S, K or Q; aa27 is N or K.

(2) - 27 "strong" peptides obtainable from such a library are preferred peptides according to various aspects of the present invention, having an amino acid sequence as follows:

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2.11 QT H TVGGVQG R QAHS LT S LF S P G A SQN *

D6 QT T TTGGQVS H ATHGLT G LF S L G P QOK *

D18 QT H TTGGSAS H QASGLT R LF S Q G P SQN *

F63 QT H VVGGQQG R QVSSLV S LF S P G A SQK *

5 G31 TT H TVGGSVA R QVHSLT G LF S P G P QOK *

L13 QT H TVGGSQA H AAHS LT R LF S P G S SQN *

M69 QT T VVGGQA R AAHGLV S LF S L G S KQN *

Z61 QT H VVGGVQG R QTSGLV G LF S P G S KQN *

R9 QT T VVGGSQS H TVRGLT S LF S P G A SQN

10 B26 TT T TTGGQAG H QAHS LT S LF S P G A SQK

B22 QT H VVGGVQS H QTSGLT S LF S P G A SQK

B35 QT H TTGGVQG H QTSRLT S LF S P G P SQN

D29 TT T VVGGQAA H QTHSLT S LF S P G A KQN

D33 TT T TTGGQQS H TVHGLV G LF S P G S KQN

15 E26 QT H TVGGVQA H TVRGLT S LF S P G S SQN

F80 QT H TTGGQAG H TASSLT G LF S P G A KQN

F19 QT T TVGGVAS H QAHS LT G LF S P G A KOK

F78 QT H TTGGQAG H QAHS LT G LF S P G A KQN

H1 QT H TTGGVVG H ATSGLT S LF S P G P SQK

20 L76 TT T TVGGQAS H QTSSLT G LF S P G S KQN

M27 QT T TTGGVAS H AAHRLT S LF S P G P QOK

M122 QT T TTGGSAS H AVSSLT G LF S P G S KQN

M129 QT T VVGGASAG H TASSLV G LF S P G S KQN

M119 TT T TVGGQAS H TTSSLT G LF S P G S QQN

R5 QT H TTGGQAS H QVSSLV S LF S P G A KQK
R6 TT T TTGGQVG H QTSGLT G LF S P G A QQN
R27 TT H VVGGSAS H AVRGLT S LF S P G S SQN

5 Further preferred peptides according to the present invention have any of the following sequences:

B14 QT T VTG_QAS H TTSSLT G LF S P G A SQK
B33 aT H aTGGQAA H STHSLT S LF S P G A SQK
10 F81 QT H VTGGSAA H QTgGLT G LF S P G P KQN
B18 QT T VVGGSAS H _VSRLT G LF S P G S SQK
L72 QT T T____AA H TTSGLT G LF S P G A KQN
D20 QT H VTG_VAG R QTSGLV S LF S P G S SQN
D30 Q_ _ _GGVQG H TTSSLV G LF S P G S QQN

15

The lower case letters are used to identify amino acid residues that vary from Formula 1, while the underlined spaces are included to signify deletions compared with Formula 1, though the flanking amino acids are of course contiguous in the relevant peptides.

These are variants of peptides obtainable from a library in accordance with the present invention, not themselves conforming with Formula I. They were identified in the course

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of the experiments identified below and originated by PCR errors during library amplification. (See Materials and Methods, "Construction of the HVR1 library".)

- 5 (3) - A "strong consensus" ("Formula II"), derived from the consensus of the highly cross-reactive peptides of (2) above.

The statistical analysis of the frequencies of aa in any position in the 27 "strong" in comparison with the frequency in 10 25 "weak" is shown in Table II, and discussed further below in the experimental section.

Formula II:

15 QT(aa3)TVGGQQS(aa11)QVHSLT(aa18)LF(aa21)(aa22)G(aa24)SQN

where: aa3 is H or T; aa11 is H or R; aa18 is G, S or R; aa21 is S; aa22 is P, L or Q; aa24 is A, S or P;

which may also be written:

20 QT H TVGGQAS H QASSLT S LF S P G A KQN

T R G L S

R Q P

Residues in italics are included because although they have low frequencies they are found in some of the best reactive

mimotopes tested (highlighted with an asterisk among the 27
"strong" peptides at II above.

The 27 mimotopes used to derive Formula II are not in it.

5

108 peptides conform to Formula II and each is an aspect
of the invention. The sequences are:

- 1 QTHTV GGQAS HQASS LTSLF SPGAK QN
- 2 QTHTV GGQAS HQASS LTSLF SPGSK QN
- 10 3 QTHTV GGQAS HQASS LTSLF SPGPK QN
- 4 QTHTV GGQAS HQASS LTSLF SLGAK QN
- 5 QTHTV GGQAS HQASS LTSLF SLGSK QN
- 6 QTHTV GGQAS HQASS LTSLF SLGPK QN
- 7 QTHTV GGQAS HQASS LTSLF SQGAK QN
- 15 8 QTHTV GGQAS HQASS LTSLF SQGSK QN
- 9 QTHTV GGQAS HQASS LTSLF SQGPK QN
- 10 QTHTV GGQAS HQASS LTGLF SPGAK QN
- 11 QTHTV GGQAS HQASS LTGLF SPGSK QN
- 12 QTHTV GGQAS HQASS LTGLF SPGPK QN
- 20 13 QTHTV GGQAS HQASS LTGLF SLGAK QN
- 14 QTHTV GGQAS HQASS LTGLF SLGSK QN
- 15 QTHTV GGQAS HQASS LTGLF SLGPK QN
- 16 QTHTV GGQAS HQASS LTGLF SQGAK QN
- 17 QTHTV GGQAS HQASS LTGLF SQGSK QN

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18 QTHTV GGQAS HQASS LTGLF SQGPK QN
19 QTHTV GGQAS HQASS LTRLF SPGAK QN
20 QTHTV GGQAS HQASS LTRLF SPGSK QN
21 QTHTV GGQAS HQASS LTRLF SPGPK QN
5 22 QTHTV GGQAS HQASS LTRLF SLGAK QN
23 QTHTV GGQAS HQASS LTRLF SLGSK QN
24 QTHTV GGQAS HQASS LTRLF SLGPK QN
25 QTHTV GGQAS HQASS LTRLF SQGAK QN
26 QTHTV GGQAS HQASS LTRLF SQGSK QN
10 27 QTHTV GGQAS HQASS LTRLF SQGPK QN
28 QTHTV GGQAS RQASS LTSLF SPGAK QN
29 QTHTV GGQAS RQASS LTSLF SPGSK QN
30 QTHTV GGQAS RQASS LTSLF SPGPK QN
31 QTHTV GGQAS RQASS LTSLF SLGAK QN
15 32 QTHTV GGQAS RQASS LTSLF SLGSK QN
33 QTHTV GGQAS RQASS LTSLF SLGPK QN
34 QTHTV GGQAS RQASS LTSLF SQGAK QN
35 QTHTV GGQAS RQASS LTSLF SQGSK QN
36 QTHTV GGQAS RQASS LTSLF SQGPK QN
20 37 QTHTV GGQAS RQASS LTGLF SPGAK QN
38 QTHTV GGQAS RQASS LTGLF SPGSK QN
39 QTHTV GGQAS RQASS LTGLF SPGPK QN
40 QTHTV GGQAS RQASS LTGLF SLGAK QN
41 QTHTV GGQAS RQASS LTGLF SLGSK QN

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42 QTHTV GGQAS RQASS LTGLF SLGPK QN
43 QTHTV GGQAS RQASS LTGLF SQGAK QN
44 QTHTV GGQAS RQASS LTGLF SQGSK QN
45 QTHTV GGQAS RQASS LTGLF SQGPK QN
5 46 QTHTV GGQAS RQASS LTRLF SPGAK QN
47 QTHTV GGQAS RQASS LTRLF SPGSK QN
48 QTHTV GGQAS RQASS LTRLF SPGPK QN
49 QTHTV GGQAS RQASS LTRLF SLGAK QN
50 QTHTV GGQAS RQASS LTRLF SLGSK QN
10 51 QTHTV GGQAS RQASS LTRLF SLGPK QN
52 QTHTV GGQAS RQASS LTRLF SQGAK QN
53 QTHTV GGQAS RQASS LTRLF SQGSK QN
54 QTHTV GGQAS RQASS LTRLF SQGPK QN
55 QTTTV GGQAS HQASS LTSLF SPGAK QN
15 56 QTTTV GGQAS HQASS LTSLF SPGSK QN
57 QTTTV GGQAS HQASS LTSLF SPGPK QN
58 QTTTV GGQAS HQASS LTSLF SLGAK QN
59 QTTTV GGQAS HQASS LTSLF SLGSK QN
60 QTTTV GGQAS HQASS LTSLF SLGPK QN
20 61 QTTTV GGQAS HQASS LTSLF SQGAK QN
62 QTTTV GGQAS HQASS LTSLF SQGSK QN
63 QTTTV GGQAS HQASS LTSLF SQGPK QN
64 QTTTV GGQAS HQASS LTGLF SPGAK QN
65 QTTTV GGQAS HQASS LTGLF SPGSK QN

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66 QTTTV GGQAS HQASS LTGLF SPGPK QN
67 QTTTV GGQAS HQASS LTGLF SLGAK QN
68 QTTTV GGQAS HQASS LTGLF SLGSK QN
69 QTTTV GGQAS HQASS LTGLF SLGPK QN
5 70 QTTTV GGQAS HQASS LTGLF SQGAK QN
71 QTTTV GGQAS HQASS LTGLF SQGSK QN
72 QTTTV GGQAS HQASS LTGLF SQGPK QN
73 QTTTV GGQAS HQASS LTRLF SPGAK QN
74 QTTTV GGQAS HQASS LTRLF SPGSK QN
10 75 QTTTV GGQAS HQASS LTRLF SPGPK QN
76 QTTTV GGQAS HQASS LTRLF SLGAK QN
77 QTTTV GGQAS HQASS LTRLF SLGSK QN
78 QTTTV GGQAS HQASS LTRLF SLGPK QN
79 QTTTV GGQAS HQASS LTRLF SQGAK QN
15 80 QTTTV GGQAS HQASS LTRLF SQGSK QN
81 QTTTV GGQAS HQASS LTRLF SQGPK QN
82 QTTTV GGQAS RQASS LTSLF SPGAK QN
83 QTTTV GGQAS RQASS LTSLF SPGSK QN
84 QTTTV GGQAS RQASS LTSLF SPGPK QN
20 85 QTTTV GGQAS RQASS LTSLF SLGAK QN
86 QTTTV GGQAS RQASS LTSLF SLGSK QN
87 QTTTV GGQAS RQASS LTSLF SLGPK QN
88 QTTTV GGQAS RQASS LTSLF SQGAK QN
89 QTTTV GGQAS RQASS LTSLF SQGSK QN

90 QTTTV GQAS RQASS LTSLF SQGPK QN
 91 QTTTV GQAS RQASS LTGLF SPGAK QN
 92 QTTTV GQAS RQASS LTGLF SPGSK QN
 93 QTTTV GQAS RQASS LTGLF SPGPK QN
 5 94 QTTTV GQAS RQASS LTGLF SLGAK QN
 95 QTTTV GQAS RQASS LTGLF SLGSK QN
 96 QTTTV GQAS RQASS LTGLF SLGPK QN
 97 QTTTV GQAS RQASS LTGLF SQGAK QN
 98 QTTTV GQAS RQASS LTGLF SQGSK QN
 10 99 QTTTV GQAS RQASS LTGLF SQGPK QN
 100 QTTTV GQAS RQASS LTRLF SPGAK QN
 101 QTTTV GQAS RQASS LTRLF SPGSK QN
 102 QTTTV GQAS RQASS LTRLF SPGPK QN
 103 QTTTV GQAS RQASS LTRLF SLGAK QN
 15 104 QTTTV GQAS RQASS LTRLF SLGSK QN
 105 QTTTV GQAS RQASS LTRLF SLGPK QN
 106 QTTTV GQAS RQASS LTRLF SQGAK QN
 107 QTTTV GQAS RQASS LTRLF SQGSK QN
 108 QTTTV GQAS RQASS LTRLF SQGPK QN

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(4) - A further library of peptides within the library of Formula I, including the sequences of Formula II, defining 2.5×10^6 sequences and conforming to the following Formula III:

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Q T H T V G G Q A S H Q A S S L T S L F S P G A K Q N
T T V T S Q G A T H G V G S S K
V V A T V R R P Q

5 A peptide according to the present invention may be
provided in a fusion with additional amino acids. Additional
amino acids may be fused at one or both of the N-terminus and
the C-terminus of the peptide. The additional amino acids may
be an amino acid sequence that is not a fragment of HCV E2
10 protein, or may be an amino acid sequence that is part of that
protein. Furthermore, a fusion including a peptide according
to the present invention may include a HCV E2/NS1 protein with
the peptide amino acid sequence in the HVR1 position, i.e. such
that the mimotope HVR1 peptide of the invention substitutes for
15 the natural HVR1 sequence. Another way of expressing this is
to refer to a "recombinant HCV E2/NS1 protein in which a
peptide of the present invention is substituted for the HVR1".
As noted below, nucleic acid encoding peptides and
polypeptides, including fusions, according to invention are
20 provided as further aspects of the invention, as is a
recombinant HCV genome including a nucleotide sequence encoding
a peptide of the invention, for instance within the E2/NS1
coding sequence to provide for production of a recombinant HCV
E2/NS1 protein in which a peptide of the invention is

substituted for the HVR1 and incorporation of the recombinant protein into an assembled HCV particle. A recombinant HCV particle including one or more peptides or polypeptides as disclosed herein is provided as a further aspect of the present
5 invention.

Generally, a peptide according to the present invention is immunogenic or able to raise an immune response on administration to an individual or includes an epitope
10 immunologically cross-reactive with an epitope of a plurality of strains of HCV.

Another aspect of the present invention provides a method of obtaining one or more peptides containing an epitope
15 immunologically cross-reactive with an epitope in the HVR1 of an HCV strain, the method including bringing into contact a library of peptides as disclosed and an antibody molecule able to bind said HVR1 of an HCV strain, and selecting one or more peptides of the library able to bind said antibody molecule.

20 The peptide or peptides selected may contain an epitope immunologically cross-reactive with the HVR1 of a plurality of strains of HCV.

Such a method may include bringing into contact a library of peptides and a plurality of antibody molecules collectively

able to bind the HVR1 of a plurality of strains of HCV. In one embodiment, said plurality of antibody molecules is derived from sera of individuals infected with HCV.

As noted, said library may be displayed on the surface of 5 bacteriophage particles, each particle containing nucleic acid encoding the peptide displayed on its surface. Following selection, nucleic acid may be taken from a bacteriophage particle displaying a said selected peptide. Nucleic acid with the sequence of nucleic acid taken from a bacteriophage 10 particle displaying a said selected peptide may be used in production of such a peptide by means of expression (using recombinant DNA technology as standard in the art and discussed further below).

A peptide with the amino acid sequence of a said selected 15 peptide may provided in isolated form, e.g. after its production by expression from encoding nucleic acid. As noted further below, one or more peptides in accordance with the present invention may be provided by peptide synthesis.

A plurality of peptides each with the amino acid sequence 20 of a different selected peptide may provided in isolated form, individually or in a mixture.

A selected peptide or selected peptides may each have an amino acid sequence according to the Formula II given above. All 108 of the different peptides according to Formula II may

be provided as a mixture, and furthermore each individually represents an aspect of the present invention. Each peptide of these 108 has a high probability of being cross-reactive with epitopes in the HVR1 of the E2/NS2 protein of a number of 5 strains of HCV, and therefore is particularly useful for obtaining antibodies or otherwise raising an immune response.

A composition according to the present invention may include a plurality of peptides obtainable from a mixture of the 108 peptides of Formula II. Such a composition may include 10 from 2 to about 20, 15, 10, 9, 8, 7, 6, 5, 4 or 3 different peptides obtainable from said mixture.

Preferred peptides which may be provided in a mixture or individually include those denoted G31, F78, R9, D6, M122 and H1 of which the amino acid sequences are shown in Figure 7(A) 15 (R9 has the same sequence as B16).

Preferred mixtures included peptides R9, F78, H1 and D6 ("MIX1"), include peptides M122 and G31 ("MIX2"), or include peptides G31, F78, R9, D6, M122 and H1 ("MIX3").

20 Immunological cross-reactivity of each peptide of the invention with the HVR1 of HCV strains can be assessed experimentally, as exemplified below. Various mixtures of these peptides may also be made and similarly tested, again as experimentally exemplified below.

Linear or branched (e.g. MAP) peptides and polypeptides (e.g. fusion molecules including a peptide as discussed) in accordance with the present invention may be made using any of a variety of techniques at the disposal of the ordinary person skilled in the art.

Linear or branched peptides may be synthesized using standard peptide chemistry such as by the common method employing Fmoc (Fluorenylmethyl-oxycarbonyl)-t-Bu (tert-butyl), as described in Atherton and Sheppard (1989), *Solid Phase Peptide Synthesis, a Practical Approach*, IRL Press, Oxford.

A convenient way of producing a peptide or polypeptide according to the present invention is to express nucleic acid encoding it, by use of the nucleic acid in an expression system.

Accordingly, the present invention also encompasses a method of making a peptide or polypeptide (as disclosed), the method including expression from nucleic acid encoding the peptide or polypeptide (generally nucleic acid according to the invention). This may conveniently be achieved by growing a host cell in culture, containing such a vector, under appropriate conditions which cause or allow expression of the polypeptide. Peptides and polypeptides may also be expressed in *in vitro* systems, such as reticulocyte lysate.

Polynucleotides encoding peptides and polypeptides according to the present invention represent further aspects of the invention.

In one aspect there is provided a polynucleotide encoding a peptide as disclosed. In a further aspect, there is provided a polynucleotide encoding a fusion as disclosed, particularly a HCV E2/NS1 protein including the amino acid sequence of a peptide of the invention in the HVR1 position. In a further aspect, there is provided a recombinant HCV genome including a nucleotide sequence encoding a peptide according to the invention or a fusion as disclosed, particularly a HCV E2/NS1 protein with the relevant peptide amino acid sequence in the HVR1 position.

In a still further aspect, a polynucleotide is provided which includes a plurality of nucleotide sequences encoding peptides or polypeptides according to the invention. This allows for production of a mixture of peptides or polypeptides in a single expression reaction.

Nucleic acid encoding a peptide or polypeptide according to the present invention may be used in nucleic acid immunisation in order to raise an immune response in a mammal, such as a human individual for a therapeutic or prophylactic purpose, or a non-human mammal for such a purpose or in order

to produce antibodies for subsequent manipulation and/or use (e.g. in diagnostic or therapeutic contexts as discussed further below.)

Nucleic acid encoding a peptide or polypeptide according to the present invention may be used in a method of gene therapy, in prevention and/or treatment of HCV infection. This requires use of suitable regulatory elements for expression and a suitable vector for deliver of the expression unit (coding sequence and regulatory elements) to host cells. A variety of vectors, both viral vectors and plasmid vectors, are known in the art, see e.g. US Patent No. 5,252,479 and WO 93/07282. In particular, a number of viruses have been used as gene transfer vectors, including papovaviruses, such as SV40, vaccinia virus, herpesviruses, including HSV and EBV, and retroviruses. Many gene therapy protocols in the prior art have used disabled murine retroviruses. A variety of adenovirus and adeno-associated viral vectors have been developed. Alternatives to viral vectors include transfer mediated by liposomes and direct DNA uptake and receptor-mediated DNA transfer.

Host cells containing nucleic acid encoding a peptide or polypeptide (or mixture thereof) according to the present invention may themselves be used in therapeutic or prophylactic treatment of individuals for or against HCV infection (i.e. therapeutic treatment of an individual with an HCV infection or

prophylactic treatment of an individual prior to HCV infection).

Nucleic acid is generally provided as DNA or RNA, though
5 may include one or more nucleotide analogues, and may be wholly
or partially synthetic. Nucleic acid molecules and vectors
according to the present invention may be provided in isolated
and/or purified form, e.g. in substantially pure or homogeneous
form. The term "isolate" may be used to reflect all these
10 possibilities. Where a DNA sequence is specified, e.g. with
reference to a figure, unless context requires otherwise the
RNA equivalent, with U substituted for T where it occurs, is
encompassed.

Where it is desired to express a peptide or polypeptide
15 from encoding nucleic acid, the nucleic acid includes
appropriate regulatory control sequences. Suitable vectors can
be chosen or constructed, containing appropriate regulatory
sequences, including promoter sequences, terminator fragments,
polyadenylation sequences, enhancer sequences, marker genes and
20 other sequences as appropriate. Vectors may be plasmids, viral
e.g. 'phage, or phagemid, as appropriate. For further details
see, for example, Molecular Cloning: a Laboratory Manual: 2nd
edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory
Press. Many known techniques and protocols for manipulation of

nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Current Protocols in Molecular Biology, 5 Ausubel et al. eds., John Wiley & Sons, 1992.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, eukaryotic cells such as mammalian and yeast, and baculovirus systems. Mammalian cell lines available 10 in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, COS cells and many others. A common, preferred bacterial host is *E. coli*.

A further aspect of the present invention provides a host 15 cell containing nucleic acid as disclosed herein. The nucleic acid of the invention may be integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques. The nucleic 20 acid may be on an extra-chromosomal vector within the cell.

A still further aspect provides a method which includes introducing the nucleic acid into a host cell. The introduction, which may (particularly for *in vitro* introduction) be generally referred to without limitation as

"transformation", may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using
5 retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage. As an alternative, direct
injection of the nucleic acid could be employed. Marker genes
10 such as antibiotic resistance or sensitivity genes may be used in identifying clones containing nucleic acid of interest, as is well known in the art.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells
15 (which may include cells actually transformed although more likely the cells will be descendants of the transformed cells) under conditions for expression of the gene, so that the encoded peptide or polypeptide is produced. If the peptide or polypeptide is expressed coupled to an appropriate signal
20 leader peptide it may be secreted from the cell into the culture medium. Following production by expression, a peptide or polypeptide may be isolated and/or purified from the host cell and/or culture medium, as the case may be, and subsequently used as desired, e.g. in the formulation of a

composition which may include one or more additional components, such as a pharmaceutical composition which includes one or more pharmaceutically acceptable excipients, vehicles or carriers (e.g. see below).

5

A peptide or polypeptide according to the present invention may be used as an immunogen or otherwise in obtaining binding antibodies. Antibodies are useful in purification and other manipulation of polypeptides and peptides, diagnostic
10 screening and therapeutic contexts, including passive immunisation. This is discussed further below.

According to a further aspect of the present invention there is provided a method of obtaining one or more antibody
15 molecules containing a binding site able to bind an epitope in the HVR1 of a plurality of HCV strains, the method including bringing into contact a population of antibody molecules and a peptide according to the present invention, and selecting one
or more antibody molecules of the population able to bind said
20 peptide.

The method may involve bringing the population of antibodies into contact with a plurality of peptides according to the invention.

As noted, the peptides may be provided in a fusion with

additional amino acids.

The peptide or peptides may be administered to a non-human mammal to bring them into contact with a population of antibody molecules produced by the mammal's immune system, then one or 5 more antibody molecules able to bind the peptide or peptides may be taken from the mammal, or cells producing such antibody molecules may be taken from the mammal.

The mammal may be sacrificed.

If cells are taken from the mammal, antibody molecules may 10 be taken from said cells or descendants thereof. Such descendants in particular may include hybridoma cells.

Instead or as well as immunising an animal, a method of obtaining antibodies as disclosed may involve displaying the population of antibody molecules on the surface of 15 bacteriophage particles, each particle containing nucleic acid encoding the antibody molecule displayed on its surface. Nucleic acid may be taken from a bacteriophage particle displaying an antibody molecule able to bind a peptide or peptides of interest, for manipulation and/or use in production 20 of the encoded antibody molecule or a derivative thereof (e.g. a fusion protein, a molecule including a constant region or other amino acids, and so on). Instead of using bacteriophage for display, ribosomes or polysomes may be used, e.g. as disclosed in US-A-5643768, US-A-5658754, WO95/11922.

Antibody molecules may be provided in isolated form, either individually or in a mixture. A plurality of antibody molecules may be provided in isolated form.

Preferred antibodies according to the invention are
5 isolated, in the sense of being free from contaminants such as antibodies able to bind other polypeptides and/or free of serum components. Monoclonal antibodies are preferred for some purposes, though polyclonal antibodies are within the scope of the present invention. Indeed, polyclonal mixtures able to
10 bind one or more peptides or polypeptides according to the present invention are preferred in some embodiments, as discussed. Thus, the present invention in a further aspect is directed to a mixture of different antibodies able to bind one or more peptides or polypeptides according to the invention.
15 Such a mixture may be provided in a composition including at least one additional component, such as a pharmaceutically acceptable excipient or vehicle.

The present invention also extends to methods of obtaining and/or raising antibodies to one or more peptides or
20 polypeptides of the invention. Such methods may include administering a peptide or polypeptide or mixture of peptides or polypeptides to a mammal in order to raise an antibody response. In a therapeutic or prophylactic context the mammal may be human or non-human. For the production of antibodies or

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antibody-producing cells to be isolated and used for any of a variety of purposes, a step of sacrificing a non-human mammal may be included. Such a non-human mammal may be for example mouse, rat, rabbit, dog, cat, pig, horse, donkey, goat, sheep, 5 camel, Old World monkey, chimpanzee or other primate.

Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to peptide or polypeptide of interest. For instance, Western blotting techniques or 10 immunoprecipitation may be used (Armitage et al, Nature, 357:80-82, 1992).

The production of polyclonal and monoclonal antibodies is well established in the art. Monoclonal antibodies can be subjected to the techniques of recombinant DNA technology to 15 produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus 20 framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB-A-2188638 or EP-A-239400. Humanised antibodies in which CDRs from a non-human source are grafted onto human framework regions, typically with the alteration of some of the framework amino acid residues, to provide

antibodies which are less immunogenic than the parent non-human antibodies, are also included within the present invention. A hybridoma producing a monoclonal antibody according to the present invention may be subject to genetic mutation or other
5 changes , which may or may not alter the binding specificity of antibodies produced. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

As an alternative or supplement to immunising a mammal with a peptide, an antibody specific for a protein may be
10 obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using bacteriophage which display functional immunoglobulin binding domains on their surfaces - for instance see WO92/01047 - or ribosomes/polysomes as noted above. The library may be naive, that is constructed
15 from sequences obtained from an organism which has not been immunised with any of the proteins (or fragments), or may be one constructed using sequences obtained from an organism which has been exposed to the antigen of interest.

Antibodies according to the present invention may be
20 modified in a number of ways. Indeed the term "antibody" should be construed as covering any binding substance having a binding domain with the required specificity. Thus the invention covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including synthetic

molecules and molecules whose shape mimics that of an antibody enabling it to bind an antigen or epitope.

Example antibody fragments, capable of binding an antigen or other binding partner are the Fab fragment consisting of the VL, VH, C1 and CH1 domains; the Fd fragment consisting of the VH and CH1 domains; the Fv fragment consisting of the VL and VH domains of a single arm of an antibody; the dAb fragment which consists of a VH domain; isolated CDR regions and F(ab')₂ fragments, a bivalent fragment including two Fab fragments linked by a disulphide bridge at the hinge region. Single chain Fv fragments are also included.

Hybridomas capable of producing antibody with desired binding characteristics are within the scope of the present invention, as are host cells, eukaryotic or prokaryotic, containing nucleic acid encoding antibodies (including antibody fragments) and capable of their expression. The invention also provides methods of production of the antibodies including growing a cell capable of producing the antibody under conditions in which the antibody is produced, and preferably secreted.

The reactivities of antibodies on a sample (e.g. in a diagnostic test) may be determined by any appropriate means. Tagging with individual reporter molecules is one possibility.

The reporter molecules may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule.

One favoured mode is by covalent linkage of each antibody with an individual fluorochrome, phosphor or laser dye with spectrally isolated absorption or emission characteristics.

10 Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine.

Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or

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biotin/streptavidin and alkaline phosphatase detection systems may be employed.

The mode of determining binding is not a feature of the present invention and those skilled in the art are able to
5 choose a suitable mode according to their preference and general knowledge.

Antibodies according to the present invention may be used in screening for the presence of a peptide or polypeptide, for
10 example in a test sample containing cells or cell lysate as discussed, and may be used in purifying and/or isolating a peptide or polypeptide according to the present invention, for instance following production of the polypeptide by expression from encoding nucleic acid therefor.

15 Antibodies are also useful in prophylaxis, by way of passive immunisation, and in therapy. Where antibodies are to be administered, it may be preferable to include a mixture of antibodies, such as antibodies collectively cross-reactive with a plurality of peptides according to the present invention.

20 Antibodies which bind a peptide in accordance with the present invention may themselves be used as immunogens in the production of anti-idiotypic antibodies. These may be used to mimic a peptide epitope in raising an immune response in an individual, e.g. for therapeutic and/or prophylactic purposes.

An antibody may be provided in a kit, which may include instructions for use of the antibody, e.g. in determining the presence of a particular substance in a test sample. One or more other reagents may be included, such as labelling 5 molecules, buffer solutions, elutants and so on. Reagents may be provided within containers which protect them from the external environment, such as a sealed vial.

Diagnostic methods make use of biological samples from 10 individuals that may contain one or more HCV strains. Examples of biological samples include fluid such as blood, plasma, serum, urine and saliva, and tissue samples.

There are various methods for determining the presence or absence in a test sample of a particular peptide or 15 polypeptide, including methods wherein the polypeptide to be detected is an antibody.

A sample may be tested for the presence of a specific binding member such as an antibody (or mixture of antibodies) directed to one or more peptides of the invention.

20 Peptides according to the present invention may be used to determine the presence or absence of antibodies against HCV strains in test samples, by assessment of binding the peptides to anti-HCV E2HVR1 antibodies if present in the sample.

In theory it may be possible to identify the presence in a

sample of a binding partner for a specific binding member such as an antibody (or mixture of antibodies) directed to one or more peptides of the invention. However, to date no-one has succeeded in isolating HCV virions from a human sample. In the
5 future, should it prove possible to identify HCV virions in human samples and/or detect such virions immunologically, peptides of the invention and particularly antibodies directed thereto will be useful in such detection.

10 For detection of antibodies to HCV, a biological or other sample may be tested by being contacted with one or more peptides of the invention under appropriate conditions for specific binding, before binding is determined, for instance using a reporter system as discussed. Where a panel of
15 peptides is used, different reporting labels may be employed for each peptide so that binding of each can be determined.

A specific binding member such as a peptide may be used to isolate and/or purify its binding partner antibody from a test sample, to allow for sequence and/or biochemical analysis of
20 the antibody. Amino acid sequencing is routine in the art using automated sequencing machines.

A typical immunoassay may involve incubating a test sample with peptides according to the invention under conditions to allow formation of immune complexes if an appropriate antibody

is present in the sample, and detecting the presence or absence of immune complex.

As noted, although not technically feasible at the moment, in principle antibodies according to the present invention may be used to determine the presence or absence of HCV strains in test samples, by assessment of binding of the antibodies to E2HVR1 epitopes if present in the sample.

A typical immunoassay may involve incubating a test sample with peptides or anti-idiotypic antibodies according to the invention under conditions to allow formation of immune complexes if an appropriate antibody is present in the sample, and detecting the presence or absence of immune complex.

A sample may be tested for the presence of an antibody directed to one or more peptides of the invention, using one or more such peptides (or polypeptide including such peptide) or one or more anti-idiotypic antibodies.

A biological or other sample may be tested by being contacted with a peptide or polypeptide or anti-idiotypic antibody under appropriate conditions for specific binding, before binding is determined, for instance using a reporter system as discussed.

The detection of formation of a binding complex in an

immunoassay in accordance with the present invention may be performed using any available technique without limitation to the scope of the invention. Some suitable techniques are described above with reference to antibody labelling. Assays 5 may involve immobilising antibody or peptide, as the case may be, on a suitable solid phase or support, such as latex particles, magnetic or non-magnetic beads, a membrane, chip, plastic, metal, silicon or glass surface, or any other suitable material at the disposal of the skilled person. Detection may 10 be qualitative or quantitative. One or more appropriate controls may be included, in accordance with standard practice.

As noted already, peptides, polypeptides, antibodies and nucleic acid in accordance with the present invention may be 15 formulated into compositions, and are useful in pharmaceutical contexts. These compositions may include, in addition to one of the above substances, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be 20 non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

Compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, 5 petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, the 10 active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride 15 Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Branched peptides, such as MAP (Tam, J.P, 1988) may be 20 used for the preparation of immunogens, either alone or linked to an appropriate carrier.

A linear peptide for use in raising an immune response may also be linked to an appropriate carrier. Various methods of coupling peptides to other molecules are known in the art,

including disulphide forming reagents (where the peptide includes a cysteine - or a cysteine is added to the peptide for this purpose), thio-ether forming coupling agents and so on. Carriers include human serum albumin (HSA), tetanus toxoid, 5 other rather large proteins that have reasonable half-lives under physiological conditions, and stable non-proteinaceous molecules such as polysaccharides and copolymers of amino acids.

An adjuvant may be included, such as alum, oil-in-water 10 emulsions or Freund's Adjuvant (Complete or Incomplete). Cytokines may be used to potentiate immunogenicity of the peptide or polypeptide composition.

Mimotope sequences may be cloned into the context of the 15 HCV envelope (E2) protein in order to use the natural folding environment for correct presentation of the epitope or epitopes to the immune system.

Naked DNA may be used for immunization (see e.g. Cohen, J, 20 1993), and one or more mimotope sequences may be cloned into suitable vectors (see e.g. Major et al., 1995). Naked DNA may be delivered using direct injection or by use of gene-guns (Yang et al., 1990) or any other suitable technique.

Whether it is a polypeptide, antibody, peptide, nucleic acid molecule, small molecule or other pharmaceutically useful compound according to the present invention that is to be given to an individual, administration may be in an immunogenic amount, that is sufficient to raise an immune (particularly antibody) response in the individual, or in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy). A prophylactic effect is sufficient to potentiate the immune response of an individual to a subsequent challenge with HCV, E2HV polypeptide, or HVR1 peptide, or to a subsequent infection with HCV, preferably in the latter case (HCV infection) to sufficient to antagonise the infection, wholly or partially. Most preferably the effect is sufficient to prevent the individual from suffering one or more clinical symptoms as a result of subsequent HCV infection, and/or protect the individual from hepatitis C. A therapeutic effect is sufficient to potentiate the immune response of an individual to pre-existing HCV infection, preferably sufficient to antagonise the infection, wholly or partially. Most preferably the effect is sufficient to ameliorate one or more clinical symptoms, and/or cure the hepatitis C and/or reduce viral titre in the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature

and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be
5 treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

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Further aspects of the invention provide methods of treatment including administration of a peptide, mixture of peptides, antibody molecule or mixture of antibody molecules, as provided, pharmaceutical compositions including such a
15 peptide, mixture of peptides, antibody molecule or mixture of antibody molecules, and use of such a peptide, mixture of peptides, antibody molecule or mixture of antibody molecules, in the manufacture of a medicament for administration, for example in a method of making a medicament or pharmaceutical
20 composition including formulating the specific binding member with a pharmaceutically acceptable excipient.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated and the availability

of alternative or additional treatments.

One aspect of the present invention provides use of a peptide as disclosed in the manufacture of a medicament for raising in a mammal antibodies able to bind HCV HVR1 epitopes.

5 Another aspect provides a method of immunising a mammal against HCV infection, the method including administering a peptide or mixture of peptides to the mammal.

A still further aspect provides a method of (passively) immunising a mammal against HCV infection, the method including
10 administering an antibody according to the invention to the mammal, or a mixture of antibodies.

Similarly, further aspects of the invention provide a method of treating a mammal with an HCV infection, the method including administering a peptide according to the invention,
15 or a mixture of peptides, or an antibody, or a mixture of antibodies, to the mammal.

The antibodies may be anti-idiotypic antibodies.

Aspects and embodiments of the present invention will now
20 be illustrated further and experimentally exemplified with reference to various figures. Further aspects and embodiments of the present invention will be apparent to those of ordinary skill in the art.

In the figures:

Figure 1(A) illustrates derivation of the consensus pattern of the 234 natural variants of the HCV HVR1 sequences used in this work. Non shaded residues within the box account 5 alone for about 80% of the observed variability. Residues are listed in decreasing order of observed frequency from top to bottom.

Figure 1(B) shows the composition in the initial HVR1 peptide library which was displayed on bacteriophage.

10 Figure 2 shows reactivity of phage pools yielded by the first round of affinity selection to antibodies present in the selecting sera. For each serum sample antibody recognition of the phage pools ($\sigma 1$, $\sigma 4R$, $\sigma 3$, $\sigma 2P$, $\sigma 2R$, $\sigma 3R$ and σN), wild type phage (wt) and the unselected library (HVR1 lib) was measured.
15 Average values (A_{405nm}) from two independent experiments have been determined.

Figure 3 shows distribution of HCV-specific phage selected from the HVR1 library as function of their frequency of reactivity with sera from infected patients. Binding is shown
20 for phage enriched by one (top panel) or two (bottom panel) cycles of affinity selection to antibodies present in twenty human sera different from those used for the selections. For each serum, average values (A_{405nm}) from two independent experiments have been determined on the selected phage and on

wild type phage. Values were considered statistically significant when differing more than $3\sigma_{\max}$ ($p < 0.003$) from the background signal observed for the wild type phage. Each histogram represents the number of phage (shown on the vertical axis) reacting with the indicated number of sera expressed as percentage over total number of tested samples (horizontal axis).

Figure 4 shows that the selected mimotopes are frequently recognized by antibodies present in human sera from HCV infected patients. Binding of the selected mimotopes to antibodies present in human sera was detected by ELISA on immobilised phage. Mimotopes' names are indicated at the top of each column. For each serum (indicated on the left of each row), average values ($A_{405\text{nm}}$) from two independent experiments have been determined. Results are expressed as the difference between the average value of the tested phagotope and that of wild type phage. Positive values are indicated in bold. Values were considered statistically significant when differing more than $3\sigma_{\max}$ ($p < 0.003$) from the background signal observed for the wild type phage. The frequency of reactivity of each mimotope and that resulting from the sum of the reactivities observed with all four mimotopes are shown at the bottom of each panel.

Figure 4(A) shows reactivity of selected mimotopes with

the panel of twenty HCV patients' sera used for the screening step.

Figure 4(B) shows reactivity of selected mimotopes with an additional panel of sera from HCV-infected viremic patients.

5 Figure 4(C) shows reactivity with sera from non viremic patients that were scored positive for anti-HCV antibodies using commercially available kits.

Figure 5 shows correlation between the S-score and the frequency of reactivity of the selected mimotopes. The
10 straight line represents the linear least square fit of the data. The correlation coefficient is 0.79.

Figure 6 shows that the selected mimotopes are antigenic mimics of a large number of naturally occurring HVR1. Antibodies from a pool of sera from HCV infected patients were
15 immunopurified on MAPs reproducing the sequence of selected mimotopes (indicated at the top of the figure). Reactivity of equal amounts of the immunopurified antibodies was measured by ELISA on a representative panel of HVR1 sequences synthesized as MAPs (indicated in the left column). Average values from
20 two independent experiments were determined. Values were considered statistically significant when two criteria were contemporarily fulfilled: (1) values were differing more than $3\sigma_{\max}$ ($p < 0.003$) from the background signal observed on two unrelated peptides; (2) values were differing more than $3\sigma_{\max}$

($p < 0.003$) from the average signal observed using ten sera from non infected individuals on each peptide representing a natural HVR1. Grey boxes indicate signals differing from those observed on the unrelated MAPs between 0.15 and 0.5 OD (405nm); 5 black boxes indicate values differing more than 0.5 OD (405nm). The level of cross-reactivity of each pool of immunopurified antibodies is indicated at the bottom of each column.

Figure 7 shows correlation between mimotope sequence and cross-reactivity.

10 Figure 7(A) shows the sequences of the mimotopes used in the analysis.

Figure 7(B) shows correlation between the S-score of the mimotopes and the cross-reactivity of immunopurified human antibodies with a panel of 43 natural HVR1 sequences. The
15 straight line represents the linear least square fit of the data. The correlation coefficient is 0.86.

Figure 8 shows that the selected mimotopes are immunogenic mimics of a large number of naturally occurring HVR1.

Reactivity of sera from mice immunised with single HVR1
20 mimotopes (Figure 8(A)) and mixtures of mimotopes (Figure 8(B)) in the form of MAP was assayed by ELISA on the panel of natural HVR1 sequences (indicated in the left column). Immunizing mimotopes are shown in the first row. MIX1 includes mimotopes R9, F78, H1 and D6; MIX2 contains M122 and G31 peptides; MIX3

is composed of all six MAPs. Titres (defined as the dilution required to obtain half maximal signal in ELISA on the homologous peptide) are shown in the second row. Sera were diluted 1:100. Average values from two independent experiments have been determined. Values were considered statistically significant when differing more than $3\sigma_{\max}$ ($p < 0.003$) from the background signal observed on two unrelated peptides. Grey boxes indicate signals differing from those observed on the unrelated MAPs between 0.15 and 0.5 OD (405nm); black boxes indicate values differing more than 0.5 OD (405nm). The level of cross-reactivity of each serum is indicated at the bottom of each column.

EXAMPLE 1 - Design and construction of a specialised phage library mimicking the HVR1 variability

A multiple sequence alignment of 234 unique HVR1 sequences extracted from the sequence databases was made to characterise the variation in residue composition at each of the N-terminal 27 positions of the HCV E2 glycoprotein. A sequence pattern emerged from this analysis (Figure 1A) allowing the definition of a degenerate consensus sequence. A synthetic repertoire of HVR1 sequences was designed to contain such conserved constraints while reproducing the observed natural variability in the remaining positions.

A "consensus-profile" accounting for approximately 80% of the total sequence variability was derived by selecting the most frequent residues at each position. When similar amino acids were present at a given position, only one was chosen as representative of the variability, preferring those residues which could more effectively form interactions. For example, in position 5 both Ser and Thr are present in the natural repertoire, but only Thr was selected to design the library (Figure 1). In some cases, a residue not present in the consensus was included in the library to better mirror the overall variability. For example, Thr was included in position 3 to account for the presence of Ser, Thr, Asn in the natural repertoire of HVR1s.

The resulting final consensus profile (Figure 1B) has a complexity of 9×10^7 very close to the upper practical limit (about 10^8) of current DNA cloning and transformation techniques. The amino acid most frequently observed in the natural repertoire was always included with the exception of position 1, (where Gln and Thr were selected although Glu is the most frequently observed amino acid). Eight positions (2, 6, 7, 16, 19, 20, 23 and 26) were kept constant given the high local sequence conservation throughout the 234 natural HVR1 variants. Noteworthy also is the total absence of negatively charged residues. With the exception of position 1, where Gln

was chosen to represent the His, Glu, Asp, Gln, Asn group, no acidic residues were present within the 80% fraction.

Qualitatively, the profile can be described as a generally more variable central region flanked by N-terminal and C-terminal 5 tails containing conserved elements.

Construction of the library proceeded by cloning a degenerated synthetic oligonucleotide as a fusion to the 5' end of the gene coding for the major coat protein (pVIII) in a phagemid vector for M13 display (see Materials and Methods).

10 About 2×10^8 independent transformants were obtained. To verify the quality and complexity of the library (HVR1 library), the inserts of fifty-six randomly chosen individual clones were sequenced. This analysis led to the following results:

- 15 (1) all clones displayed different sequences;
- (2) 63% of the clones contained full-length inserts while the remaining ones had small deletions;
- (3) none of the sequenced clones encoded for peptides corresponding to known HVR1 from viral isolates, searched on 15
- 20 March 1998.

From these data it was inferred that the library has a complexity close to the number of individual transformants.

EXAMPLE 2 - Identification of HVR1 mimotopes frequently

reacting with HCV patients' sera

The more complex and diverse the repertoire of antibodies used for the selection, the higher should be the probability to enrich phage recognised by many different antibodies against
5 HVR1 epitopes. Sera from chronically infected, viremic patients appear to meet these requirements as these individuals have a rather long history of viral persistence, during which a large number of HCV variants have been generated and have challenged the immune system, presumably leading to the
10 accumulation of a highly heterogeneous population of anti-HVR1 antibodies.

Eight sera from chronic patients infected by viruses of five different genotypes: 1a, 1b, 2a, 2b, 3a (Simmonds et al., 1993) were used to perform six affinity selections of the HVR1
15 library (Table 1). As control, a serum from a non infected individual was also used. Pools of phage obtained from all seven selections were amplified and tested for their reactivity to each of the selecting sera in ELISA. The results of this experiment showed a significant enrichment of phage recognised
20 by the selector antibodies, as evidenced by the increase in reactivity with respect to the unselected library (Figure 2). In most cases, phage pools enriched by HCV sera reacted with more than one patient's serum. Peptides recognised by antibodies unrelated to HCV infection were also enriched from

the library. In fact, the pool of phage selected with the control serum has a higher reactivity with this serum than the unselected library (Figure 2). However, patients' sera drove selection toward HCV-related mimotopes as no reactivity to 5 phage pools enriched by HCV sera was detected using sera from healthy individuals (Figure 2 and data not shown).

To gain insight into the frequency of reactivity of the selected mimotopes with different patients' sera, forty individual clones from two pools (4R and 2R, Table 1) were 10 randomly chosen and tested for their reactivity in ELISA with a panel of twenty sera from HCV infected patients different from those used for the selection. An equivalent number of sera from non-infected healthy controls were used to assess the specificity for anti-HCV antibodies. Twenty-four clones turned 15 out to be HCV-specific. Their distribution as a function of their frequency of reactivity with patients' sera is reported in Figure 3 (upper panel). Among them, phage reacting with more than one serum were identified; some of these were recognised by up to 55% of the tested sera.

20 To further improve the isolation of mimotopes reacting with many different anti-HVR1 antibodies, the enriched phage pools were subjected to a second round of affinity selection using patients' sera different from those used for the first round. In this way nine new pools were generated (Table 1) and

analysed by ELISA. As before, a general increase in reactivity with the selector antibodies was observed. In addition, all second round phage pools reacted more frequently than those selected in the first round with a panel of sera from HCV-
5 infected patients different from those used for either selection, reflecting a higher recognition frequency of the isolated peptides. This was confirmed by comparing the reactivity with HCV sera of clones randomly chosen among those eluted after one round of affinity selection (Figure 3, upper
10 panel) and those obtained by re-selecting them with a second different serum (Figure 3, lower panel). Not only the frequency, but also the distribution of reactivity appeared to be significantly different after the second selection step. While recognition of phage from the first selection appears to
15 be rather scattered, clones isolated through two rounds of selection show a bell-shaped distribution of their frequency of reactivity with an average value of 60% (Figure 3, lower panel), indicating that the whole phage population had indeed acquired more of the desired binding properties. It was
20 decided to omit additional selection cycles to avoid introduction of a bias toward biologically favoured phage during amplification.

A total of one hundred and seventy one clones reacting exclusively with HCV sera were identified by screening all

second-round pools. Their distribution as a function of the recognition frequency by HCV sera mirrored that of the subset displayed in Figure 3, lower panel, with the best clones reacting with 80% of the tested samples. More importantly, the profiles of reactivity of the selected mimotopes highlight another relevant feature. Despite their quantitative similar overall frequency of recognition by the HCV sera, different clones display a characteristic pattern of reactivity with the net result that few mimotopes can score for the presence of anti-HVR1 antibodies in all tested sera (Figure 4A).

Next, it was verified whether the observed high frequency of recognition by HCV sera was limited to the tested patients' population or whether it reflected an intrinsic property of the selected mimotopes. For this purpose another set of sera from infected patients was assayed by ELISA revealing that both the frequency of reactivity of each individual phage and the total coverage of the sera remained unaltered (Figure 4B).

HCV infected individuals who have resolved the infection most likely came in contact with a lower number of viral variants and presumably developed a narrower spectrum of variant-specific anti-HVR1 antibodies than chronically infected patients. This is supported by the finding that sera from the former population react with synthetic peptides reproducing the HVR1 of natural isolates much more rarely than those of

chronically infected viremic patients (Scarselli et al., 1995). Therefore, non viremic sera could constitute a better and more stringent test for assaying the cross-reactivity of HVR1 mimotopes with different anti-HVR1 antibodies. Some of the
5 selected mimotopes were thus tested against forty-one samples from HCV seropositive individuals who were repeatedly found negative for the presence of viral RNA in the blood. Again, the mimotopes reacted with many of these sera albeit at a lower frequency than that observed with sera from viremic patients
10 (compare Figures 4(A), 4(B) and 4(C)). These data provide an indication of the ability of the selected mimotopes to cross-react with a large number of different anti-HVR1 antibodies.

*EXAMPLE 3 - Determination of a relationship between the
15 sequence of the selected HVR1 mimotopes and their frequency of reactivity with HCV sera.*

The inventors wished to verify whether the amino acid sequence of the selected clones correlates with their frequency of reactivity. No obvious pattern arises from a visual
20 comparison of the sequences so it was decided to analyse separately the sequence patterns of the least and most frequently reacting clones.

Defined as "weak" were the 24 clones that only reacted with less than 3 sera and defined as "strong" were the 27